Crystal Structures of Bovine Milk Xanthine Dehydrogenase and Xanthine Oxidase: Structure-Based Mechanism of Conversion

C. Enroth, B. Eger (U. of Toronto), K. Okamoto, T. Nishino, T. Nishino (Nippon Medical School), and E. Pai (U of T)

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Introduction: Milk xanthine oxidase is an archetypal enzyme, which was originally described as aldehyde oxidase in 1902 and has since served as a benchmark for the whole class of complex metalloflavoproteins. Xanthine oxidoreductase enzymes have been isolated from a wide range of organisms, from bacteria to man, and catalyze the hydroxylation of a wide variety of purine, pyrimidine, pterin, and aldehyde substrates. All of these proteins have similar molecular weights and composition of redox centers. The mammalian enzymes, which catalyze the hydroxylation of hypoxanthine and xanthine, the last two steps in the formation of urate, are synthesized as the dehydrogenase form (XDH) and exist mostly as such in the cell but can be readily converted to the oxidase form (XO) by oxidation of sulfhydryl residues or by proteolysis. XDH shows a preference for NAD^+ reduction at the FAD reaction site, while XO fails to react with NAD^+ and exclusively uses dioxygen as its substrate leading to the formation of superoxide anion and hydrogen peroxide. The enzyme is a target of drugs against gout and hyperuricemia and the conversion of XDH to XO is of major interest as it has been implicated in diseases characterized by oxygen radical-induced tissue damage, such as postischemic reperfusion injury. Recent work suggests that XO might also be associated with blood pressure regulation.

The active form of the enzyme is that of a homodimer of molecular mass 290 kDa, with each of the monomers acting independently in catalysis. Each subunit contains one molybdopterin cofactor, two spectroscopically distinct [2Fe-2S] centers, and one flavin adenine dinucleotide (FAD) cofactor. The oxidation of xanthine takes place at the molybdopterin center (Mo-pt). The reduction of NAD^+ occurs through FAD.

Methods and Materials: We were able to grow diffraction-quality crystals of both the XDH and XO forms in complex with the inhibitor salicylate. At 100 K, native data sets were collected from flash-frozen crystals to 2.1 Å resolution for XDH and to 2.5 Å for XO, respectively. MAD data were measured on XDH crystals at three wavelengths near the iron edge. The structures of XDH and XO were solved by a combination of molecular replacement and anomalous phasing from the iron atoms in the Fe/S centers of XDH.

Results: The overall dimensions of the dimeric enzyme are 155 Å x 90 Å x 70 Å. It has a butterfly shape with the dimer interface on the smaller side of the elongated subunits. Each subunit has overall dimensions of 100 Å x 90 Å x 70 Å. The monomer can be divided into three domains (Fig. 1). The small N-terminal domain contains both iron-sulfur cofactors and is connected to the second, FAD-binding domain by a long segment, in which no electron density could be seen for residues 166 to 191. The FAD domain is connected to the third domain by another linker segment, which is also partially disardered. The large third domain sequesters the Mo-pterin cofactor close to the interfaces of the Fe/S- and FAD-binding domains. The FAD active site is the part of the enzyme that shows the largest changes when XDH is converted to XO. A highly charged loop passing opposite the *si*-side of the flavin ring moves up to 20 Å, drastically changing the electrostatic potential of the flavin environment.

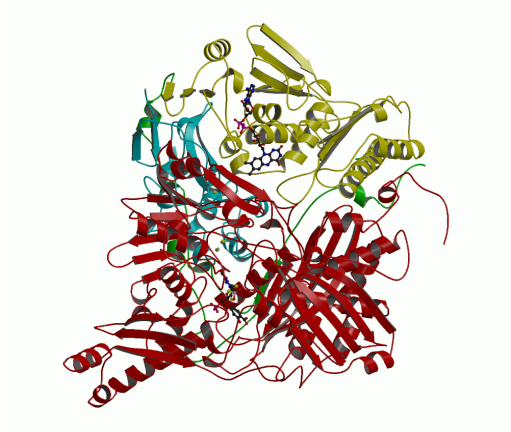

Figure 1. Ribbon diagram of one subunit of bovine milk xanthine dehydrogenase. Its domains and linkers are color-coded, the cofactors are included.

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